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(54) **Cell preservative solution**

Konservierungslösung für Zellen

Solution pour la préservation des cellules

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Description

[0001] This invention relates to a solution and method for preservation of cells at ambient temperatures. The solution and method provide rapid fixation of live cells for subsequent analysis.

[0002] It is known in the clinical and research arenas that preservation of cell samples for subsequent analysis is desirable. From a diagnostic standpoint, a specimen is most valuable when it is fresh. The more time that elapses between collection of a specimen and its fixation on a slide or other matrix, the less integrity is retained. Depriving cells of the physiologic conditions of its donor for long periods of time, i.e., minutes, allows autolysis to begin.

[0003] In a clinical setting it is often necessary to take samples, e.g., vaginal cells or muscle cells from a patient, which are later stained for histology analysis. Such histochemical staining has unquestionable value in the interpretation and study of cell physiology and pathology, however the staining cannot usually be performed simultaneous with the sampling. It is often desirable to perform a biopsy on a patient at one time, and to perform cytological or histological analysis of the collected cells or tissue at a different time. Cells often lose integrity in that interim period, thus diminishing the value of the subsequent analysis.

[0004] Several types of saline, or balanced salt, solutions are commercially available for preserving cell specimens in the interim between sampling and fixation and/or analysis. A few of these solutions includes Hanks' balanced salt solution, a minimal essential (MEM) tissue culture medium, Polysal®, and normal saline. The high cost of some medium, such as Hanks' and MEM, prohibits its routine use.

[0005] Polysal®, available from Cutter Biologicals, Emeryville, California, is a balanced polyionic electrolyte solution containing sodium chloride, calcium, and magnesium at a physiologically equivalent concentration to normal human plasma. Although an adequate saline solution for relatively short-term storage, it neither inhibits bacterial growth nor enables extended ambient storage.

[0006] Hanks' BSS is a modified Ringer solution. It is designed to maintain osmotic pressure within physiologic limits, maintain optimal pH range by including buffer systems, and provides an adequate concentration of inorganic ions for normal cell metabolism. This solution includes a glucose energy source. However, cells lose viability after an exposure of over twenty minutes, which affects cytopathologic analysis.

[0007] Many types of clinical tissue and cell samples contain extraneous proteins which interfere with subsequent staining and analysis. Placement of specimen cells in a saline solution does not address some auxiliary problems with such sample integrity. Extended preservation of specimens often results in bacteria growth, which is also nurtured by the balance of prior art normal or augmented saline solutions.

[0008] Accordingly, it is an object of the present invention to provide a cell fixing solution and process which preserves cells and tissue for subsequent cytological or histological analysis.

[0009] This object is achieved by a solution according to claim 1 and a method according to claim 13.

[0010] This invention generally relates to a solution and method for preservation of cells and tissue at ambient temperatures. The solution is an alcohol buffer solution for in vitro preservation of the nuclear morphology of mammalian cells at ambient temperatures following biopsy, and prior to staining or other forms of analysis. In one embodiment, the preservation solution provides a medium for relatively long-term ambient preservation. In another embodiment, the preservation solution provides a medium for transportation and removal of undesired protein from the sample solution.

[0011] More specifically, a preservative solution according to the invention has water-miscible alcohol, in combination with a buffering agent. The alcohol constituent is present in an amount sufficient to fix sample cells or tissue. The buffering agent is one which maintains the pH of the solution within a range of between about four to about seven for the duration of preservation.

[0012] In a preferred embodiment of the invention, the alcohol is one from the group consisting of ethanol and methanol. In one embodiment, the solution comprises methanol, magnesium acetate, calcium acetate, potassium chloride, and sodium chloride. The alcohol constitutes approximately 20 percent of the solution, about 0.1% sodium chloride, 10 mM potassium chloride, 2 mM calcium acetate, and 1 mM magnesium acetate.

[0013] In an illustrative practice of the method of the invention, a sample of mammalian cells is provided and, within a predetermined or specified time frame following biopsy, the cells are suspended in a preservation solution of the type described above. In one embodiment of the invention, the suspended cells can be preserved at an ambient temperature in the range of from about 4° to about 38° centigrade (C) for a period of at least approximately three weeks. Throughout this time, the cells retain sufficient structure to enable staining without a significant loss of integrity.

[0014] In another illustrative practice of the method of the invention, a sample of mammalian cells is provided and, within a specified time frame following biopsy, the cells are suspended in a preservation solution of the invention. In that embodiment of the invention, the sample is placed in the preservation solution to remove undesired protein from the cell sample. The clean sample may then be transported in the inventive solution for subsequent analysis and/or storage.

[0015] The present invention generally relates to an alcohol-buffer solution for the preservation of mammalian cells in suspension at ambient temperature. The solution enhances maintenance of the nuclear structure of the cells, in that it maintains cell membranes intact for

subsequent cytological staining. The solution also effectively destroys microbial pathogens in a sample, and inhibits retroviral activity. In one form, the solution removes undesired protein material from the sample.

[0016] More particularly, the cell-preservation solution of the invention includes a combination of an alcohol, and a buffer that maintains the solution at a pH of between about four to seven for the duration of the preservation time.

[0017] In one embodiment, the preservation time for cells in the present solution, at ambient temperature (approximately 37°C), is approximately three weeks. This duration may be altered by both the stored age of the solution prior to ambient cell suspension, the amount of time between cell sampling and cell suspension, and the alcohol content. For example, if the solution has been stored for a significant length of time, in either a refrigerated state or an ambient state, then the remaining cell-preserving viability of the solution may be limited.

[0018] In a preferred embodiment, the alcohol is methanol. Other alcohols which may be used include isopropanol and ethanol among others. This alcohol consituent maintains cell DNA integrity and retains the detail of the cell nucleus for subsequent cytological staining and analysis.

[0019] In one embodiment of the invention, the alcohol is present in an amount of approximately 45% to 55% by solution. Solutions containing 60% or above of the alcohol constituent tend to exhibit clumping, or coagulation, which interferes with the subsequent ability to effectively stain the sample cells. Conversely, if the concentration of alcohol in this embodiment is at 40% or below, the cells are not sufficiently fixed for relatively long-term preservation, causing the cells to degrade over time. For this embodiment, the solution contains approximately 50% methanol, by solution.

[0020] In another embodiment of the invention, the alcohol is present in an amount approximately 20 percent by solution. While this concentration of alcohol, as noted above, does not enable long-term preservation, (i.e., over two days), it does sufficiently fix cells for subsequent analysis. Alternatively, the cells may be transferred from this 20% embodiment solution to a 50% embodiment of the solution, for subsequent long-term preservation prior to analysis.

[0021] The buffer used in the inventive solution has a large buffering range to accommodate for the change in pH resulting from autolytic by-products from the sample cells suspended in the solution. For example, as cervical cells age, they release autolytic by-products that alter the pH balance of the suspension solution. In addition, the preservation of different cell types may require solutions of different acidity and within different pH ranges. Accordingly, a solution having a broad buffering range can be used for a wide range of cell types and is optimal for the solution of the invention. Exemplary cells for which this solution can be used include cervical cells, white blood cells, bronchial cells, and sputum, among others.

[0022] Accordingly, a preferred buffer is an acetate buffer, such as sodium acetate, magnesium acetate, calcium acetate, and combinations thereof. While other buffers, such as phosphate or Tris buffers, may be used in the present solution, the effective buffering range of these buffers is deemed to be not as broad at the desired pH as that of acetate.

[0023] In practicing the method of the invention, a cell sample is obtained from a patient or other cell source. A preservation solution of the type described above is placed either in a vial, on a welled slide, or on an appropriate membrane. The collected cells are then placed in the solution, preferably within one minute following collection. The sooner the collected cells are placed in the preservative solution, the longer the cells can be preserved at ambient temperature suspended in the solution, since the trauma to the cells is minimized.

[0024] Following preservation and/or protein removal, when the cells are to be stained or otherwise analyzed, a device can be used to remove suspended cells, along with the suspension preservation medium, and place them on a slide or other appropriate surface for further processing.

Example 1

[0025] An embodiment of the invention includes the following formulation:

1 mM magnesium acetate
2 mM calcium acetate
10 mM potassium chloride
0.1% sodium chloride
20% methanol

In this formulation, the function of the calcium and magnesium ions is the preservation of nuclear morphology of cytologically significant cells. The acetate is present as a buffer that will both stabilize the pH of the solution, and not form precipitates of calcium and magnesium. Such precipitation would happen with a phosphate buffer. The sodium and potassium salts are present to help stabilize the cells and prevent precipitation and coagulation of hemoglobin and other serum proteins. The methanol is present to aid in the lysing of red blood cells, to act as a preservative against bacterial growth, and to help preserve cytologically significant cells.

Claims

1. An aqueous alcohol-buffer solution for preserving in vitro the nuclear morphology of mammalian cells in a cell containing sample, the solution comprising:

   a water-miscible alcohol selected from the group consisting of methanol, ethanol and iso-

propanol wherein the alcohol constitutes 20% to no greater than 60% of the solution;
magnesium and calcium ions in an amount sufficient to preserve nuclear morphology of mammalian cells in the sample;
a buffering agent which maintains the solution at a pH in a range of 4 to 7.

2. The solution of claim 1, wherein the alcohol constitutes 20 % of the solution.

3. The solution of claim 1 or 2, wherein the buffering agent in an acetate buffer.

4. The solution of any of claims 1 or 3, comprising magnesium acetate and calcium acetate for providing the magnesium and calcium ions and the buffering agent.

5. The solution of any one of claims 1 to 4, comprising 2 mM calcium acetate.

6. The solution of any one of claims 1 to 5, comprising 1 mM magnesium acetate.

7. The solution of any one of claims 1 to 6, comprising potassium ions in an amount sufficient to reduce precipitation or coagulation of hemoglobin, if present, in the sample.

8. The solution of any one of claims 1 to 7, comprising sodium ions in an amount sufficient to reduce precipitation or coagulation of hemoglobin, if present, in the sample.

9. The solution of claim 7 or 8, comprising methanol as the water miscible alcohol and sodium chloride and potassium chloride for providing the potassium and sodium ions.

10. The solution of claim 9, comprising 0.1% sodium chloride.

11. The solution of claim 9 or 10, comprising 10 mM potassium chloride.

12. A method of preserving nuclear morphology of mammalian cells in vitro, the method comprising combining the cells with a solution of any one of claims 1 to 11.

13. The method of claim 12, further comprising the step of maintaining the cell containing solution at a temperature in the range of 4°C to 38°C.

Patentansprüche

1. Eine wässrige Alkohol-Pufferlösung zur Konservierung der Zellkernmorphologie von Säugerzellen in einer Zell-enthaltenden Probe in vitro, wobei die Lösung umfasst:

einen mit Wasser mischbaren Alkohol, der aus der Gruppe bestehend aus Methanol, Ethanol und Isopropanol ausgewählt ist, wobei der Alkohol in einem Anteil von 20 % bis nicht mehr als 60 % in der Lösung enthalten ist;
Magnesium- und Calciumionen in einer Menge, die ausreichend ist, um die Zellkernmorphologie von Säugerzellen in der Probe zu konservieren;
ein Puffermittel, das die Lösung bei einem pH-Wert in einem Bereich von 4 bis 7 hält.

2. Lösung nach Anspruch 1, bei welcher der Alkohol in einem Anteil von 20 % in der Lösung enthalten ist.

3. Lösung nach Anspruch 1 oder 2, bei der das Puffermittel ein Acetatpuffer ist.

4. Lösung nach einem der Ansprüche 1 bis 3, die Magnesiumacetat und Calciumacetat zur Bereitstellung der Magnesium- und Calciumionen und das Puffermittel umfasst.

5. Lösung nach einem der Ansprüche 1 bis 4, die 2 mM Calciumacetat umfasst.

6. Lösung nach einem der Ansprüche 1 bis 5, die 1 mM Magnesiumacetat umfasst.

7. Lösung nach einem der Ansprüche 1 bis 6, die Kaliumionen in einer Menge umfasst, die ausreichend ist, um die Ausfällung oder Koagulation von Hämoglobin, falls dieses vorliegt, in der Probe zu vermindern.

8. Lösung nach einem der Ansprüche 1 bis 7, die Natriumionen in einer Menge umfasst, die ausreichend ist, um die Ausfällung oder Koagulation von Hämoglobin, falls dieses vorliegt, in der Probe zu vermindern.

9. Lösung nach Anspruch 7 oder 8, die Methanol als mit Wasser mischbaren Alkohol und Natriumchlorid und Kaliumchlorid zur Bereitstellung der Kalium- und Natriumionen umfasst.

10. Lösung nach Anspruch 9, die 0,1 % Natriumchlorid umfasst.

11. Lösung nach Anspruch 9 oder 10, die 10 mM Kaliumchlorid umfasst.

12. Ein Verfahren zur Konservierung der Zellkernmorphologie von Säugerzellen in vitro, wobei das Verfahren das Zusammenbringen der Zellen mit einer Lösung nach einem der Ansprüche 1 bis 11 umfasst.

13. Verfahren nach Anspruch 12, das ferner den Schritt des Haltens der Zell-enthaltenden Lösung bei einer Temperatur im Bereich von 4°C bis 38°C umfasst.

Revendications

1. Solution tampon alcoolique aqueuse pour la préservation *in vitro* de la morphologie nucléaire de cellules mammifères dans un échantillon contenant une cellule, la solution comprenant :

   un alcool hydrosoluble sélectionné parmi le groupe comprenant le méthanol, l'éthanol et l'isopropanol dans lequel l'alcool constitue entre 20 % et pas plus de 60 % de la solution ;
   des ions magnésium et des ions calcium en une quantité suffisante pour préserver la morphologie nucléaire des cellules mammifères dans l'échantillon ;
   un agent tampon qui maintient la solution à un pH compris dans la plage allant de 4 à 7.

2. Solution selon la revendication 1, dans laquelle l'alcool constitue 20 % de la solution.

3. Solution selon la revendication 1 ou 2, dans laquelle l'agent tampon est un tampon d'acétate.

4. Solution selon l'une quelconque des revendications 1 à 3, comprenant de l'acétate de magnésium et de l'acétate de calcium dans le but de fournir les ions magnésium et les ions calcium ainsi que l'agent tampon.

5. Solution selon l'une quelconque des revendications 1 à 4, comprenant 2 mM d'acétate de calcium.

6. Solution selon l'une quelconque des revendications 1 à 5, comprenant 1 mM d'acétate de magnésium.

7. Solution selon l'une quelconque des revendications 1 à 6, comprenant des ions potassium en une quantité suffisante pour réduire la précipitation ou la coagulation de l'hémoglobine, si elle est présente, dans l'échantillon.

8. Solution selon l'une quelconque des revendications 1 à 7, comprenant des ions sodium en une quantité suffisante pour réduire la précipitation ou la coagulation de l'hémoglobine, si elle est présente, dans l'échantillon.

9. Solution selon la revendication 7 ou 8, comprenant le méthanol en tant qu'alcool hydrosoluble et le chlorure de sodium ainsi que le chlorure de potassium dans le but de fournir les ions potassium et les ions sodium.

10. Solution selon la revendication 9, comprenant 0,1 % de chlorure de sodium.

11. Solution selon la revendication 9 ou 10, comprenant 10 mM de chlorure de potassium.

12. Procédé de préservation de la morphologie nucléaire de cellules mammifères *in vitro*, le procédé comprenant la combinaison des cellules avec une solution selon l'une quelconque des revendications 1 à 11.

13. Procédé selon la revendication 12, comprenant en outre l'étape consistant à maintenir la solution contenant la cellule à une température comprise dans la plage allant de 4°C à 38°C.